Formaldehyde biodegradation and its effect on the denitrification process

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Abstract

Simultaneous formaldehyde biodegradation and denitrification in batch assays and in a continuous lab-scale reactor were studied. In batch assays, initial biodegradation rates between 0.7 and 3.3 g CH_2O g VSS^{-1} d^{-1} were obtained at formaldehyde concentrations between 300 and 2150 mg l^{-1}. The denitrification process was affected by the presence of formaldehyde. The nitrite accumulation increased with the initial formaldehyde concentration. In the continuous reactor, removal efficiencies above 98.5% were obtained at formaldehyde loading rates between 0.37 and 2.96 kg COD m^{-3} d^{-1} (625 - 5000 mg CH_2O l^{-1}). Formaldehyde removal led to the appearance of methanol and formic acid in the medium. Denitrification process was almost complete (around 99.7%) at nitrogen loading rates up to 0.44kg $N\text{-}NO_3^-$ m^{-3} d^{-1}. Nitrite occasionally appeared in the effluent at concentrations less than 2.9 mg l^{-1}. The composition of the biogas indicated that denitrification and methanogenesis occurred simultaneously in the same unit.

Keywords

Formaldehyde, biodegradation, denitrification

Introduction

Formaldehyde is a common compound in the chemical industry, used in a wide variety of processes and frequently found in wastewaters and waste gases [1]. Because of its toxicity, formaldehyde is often used as an active ingredient in preservatives and disinfectant agents to inhibit microbial activity. Nevertheless, formaldehyde is known to be biodegradable in both aerobic [2, 3, 4] and anaerobic systems [5, 6, 7]. Few studies have been published on the biological removal of formaldehyde under anoxic conditions [8, 9, 10].

Wastewaters from aminoplastic resin producing industries are characterized by the presence of high levels of nitrogen compounds (56 - 1462 mg TKN l-1) and organic matter, mainly formaldehyde (7 - 2711 mg CH_2O l^{-1}) [11]. Therefore, the treatment of these wastewaters requires the simultaneous removal of nitrogen compounds and organic matter, which can be undertaken by biological processes. It is necessary to attain both formaldehyde biodegradation and nitrogen removal by nitrification and denitrification. During the nitrification step, ammonium is oxidized to nitrate under aerobic conditions; and during the denitrification step, nitrate is reduced to molecular nitrogen under anoxic conditions. For denitrification to take place, a source of organic carbon is required, which is the electron donor to be oxidised by nitrate. In the presence of a toxic compound such as formaldehyde, the biological nitrogen removal may be inhibited. In previous researches, the simultaneous nitrification and formaldehyde biodegradation was studied in aerobic batch assays [11] and in an activated sludge unit [12].

The aim of the present research was to study the simultaneous formaldehyde biodegradation and denitrification at lab-scale, first in anoxic batch assays and then in a continuous anoxic reactor. The biodegradability of formaldehyde and its effect on the denitrification process were investigated. The results can be used to optimize the operation of an industrial-scale wastewater treatment plant treating effluents which contain these compounds.

Materials and methods

Analytical Methods

Formaldehyde was analyzed spectrophotometrically according to the Hantzch reaction [13], using a Perkin Elmer Lambda 11 UV/Vis spectrophotometer. Methanol was measured using a Hewlett Packard 5890-II gas chromatograph equipped with a Nukol column (30 m x 0.25 mm ID) and a flame ionization detector. Nitrogen (1.5 ml min^{-1}) was utilised as carrier gas. Injector and detector temperatures were 250 and 270ºC, respectively. Formic acid was determined using a Hewlett Packard 1100 liquid chromatograph equipped with a C-18 ODS column (25 cm x 4 mm ID) and a UV diode-array detector. The mobile phase was acetonitrile:phosphoric acid (80:20) at a flow rate of 1 ml min^{-1}. Detection was performed at 210 nm.

Nitrite and nitrate were analyzed by capillary electrophoresis using a Hewlett Packard 3DCE system with a microcapillary tube of fused silica (40 cm x 50 x m ID). UV detection was undertaken at a wavelength of 214 nm and 450 nm as reference. The biogas composition (N^2, CH^4, CO^2 and N2O) was analyzed on a Hewlett Packard 5890-II gas chromatograph equipped with a Porapack Q W80/100 column (2 m x 1/8" ID) and a thermal conductivity detector. Helium (15 ml min^{-1}) was utilised as carrier gas. Injector, oven and detector temperatures were 90, 25 and 100ºC, respectively. pH, chemical oxygen demand (COD) and volatile suspended solids (VSS) were evaluated according to *Standard Methods* [14].

Batch Assays

After some preliminary experiments that allowed defining the most appropriate operating conditions, anoxic batch assays were undertaken in 300 ml vials filled with 250 ml medium. Each flask was inoculated with 2 g VSS l^{-1}, using sludge obtained from the anoxic chamber of the full-scale wastewater treatment plant of a synthetic resin producing factory. The initial pH was adjusted to 7.5; sodium bicarbonate was used as pH buffer. The medium was supplemented with 2.5 ml nutrient solution composed of (g l^{-1}): $CaCl_2 \cdot 2H_2O$ 1.00, $FeSO_4 \cdot 7H_2O$ 0.50, $MnCl_2 \cdot 4H_2O$ 0.25, $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ 0.05, $CuSO_4 \cdot 5H_2O$ 0.05, $MgSO_4 \cdot 7H_2O$ 2.40 and $CoCl_2 \cdot 6H_2O$ 0.001. Ammonium chloride and potassium phosphate monobasic were added in order to obtain a COD/N/P ratio of 200/5/1. Potassium nitrate (250 mg N l^{-1})
and formaldehyde (from 300 to 2150 mg l^{-1}) were added in order to study the denitrification process and formaldehyde biodegradation. Finally, the flasks were sealed and nitrogen gas was passed through the head space for 5 minutes in order to remove oxygen. Assays were performed in a thermostatic chamber at 20ºC and with constant shaking at 200 rpm.

Continuous Reactor

An anoxic upflow sludge blanket reactor, made of glass with a length of 45 cm, an inner diameter of 5.5 cm and an effective volume of 0.92 l, was used for continuous assays [15]. The system was provided with a liquid displacement biogas measurement device [16].
The reactor was inoculated with 8 g VSS l^{-1} of anoxic sludge from the full-scale wastewater treatment plant of a synthetic resin producing factory. The hydraulic retention time was 1.8 days. The influent consisted of a synthetic solution containing formaldehyde, potassium nitrate, potassium phosphate buffer (pH around 7.0) and 10 ml l^{-1} of the nutrient solution mentioned before. Formaldehyde and nitrate concentrations in the influent were increased stepwise from 625 to 5000 mg l^{-1} and 100 to 800 mg l^{-1}, respectively; maintaining the COD/N ratio at 6.7.

Results and discussion

Batch Assays

Biodegradation of formaldehyde as the single carbon source

Formaldehyde biodegradation was investigated in batch assays at initial concentrations between 300 and 2150 mg l^{-1} (Figure 1). The data show that high formaldehyde concentrations could be removed in anoxic conditions using that pollutant as the single carbon source. The initial substrate consumption rates (rs) depicted against initial formaldehyde concentrations are shown in Figure 2. The results indicate the relatively fast complete removal of high formaldehyde concentrations. Initial substrate consumption rates between 0.7 and 3.3 g CH_2O g VSS^{-1} d^{-1} were obtained. The increase in the initial formaldehyde concentration caused a steady increase in its initial biodegradation rate, suggesting first order kinetic (r^2 = 0.9902) and basically no inhibition effect.The first order rate constant was 0.12 h^{-1}.
In similar batch assays performed in our laboratory in a nitrifying medium, formaldehyde was also completelybiodegraded [11]. Higher initial formaldehyde biodegradationrates were obtained in aerobic assays, reaching a value of 4.9 g CH2O g VSS^{-1} d^{-1} for 2150 mg l^{-1} formaldehyde. A first order kinetic was also observed under aerobic conditions, with a rate constant of 0.31 h^{-1}.

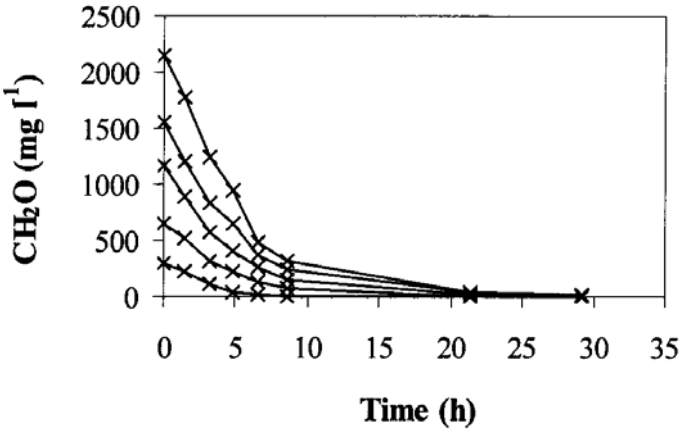

Figure 1. Formaldehyde biodegradation at different concentrations versus time

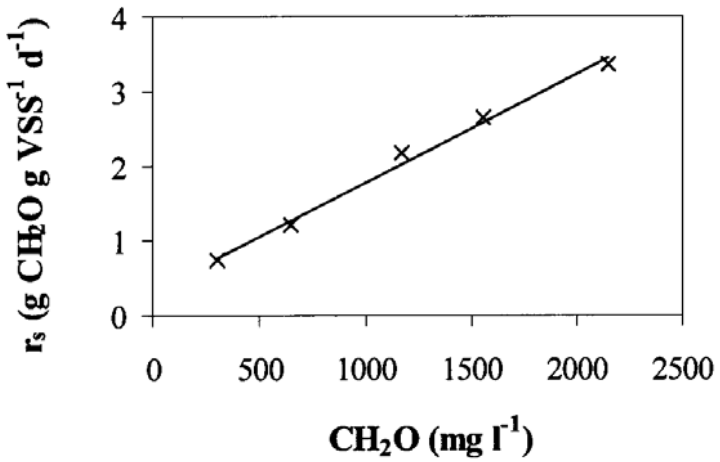

Figure 2. Initial substrate consumption rates (r_s) depicted against initial formaldehyde concentrations.

Denitrification process in the presence of formaldehyde

Biological denitrification in the presence of formaldehyde was also studied in batch assays. The initial nitrate concentration was 250 mg N l^{-1} and the initial formaldehyde concentration ranged from 300 to 2150 mg l^{-1}. The variation of nitrite and nitrate concentrations is presented in Figure 3. Complete denitrification did not take place in any case. In all the assays, except at the lowest formaldehyde concentration (300mg l^{-1}), nitrite was detected as intermediate of the denitrification process. In assays with 300 mg l^{-1} formaldehyde, the COD/N ratio (1.3) was lower than the theoretical value required for the denitrification percentage that was achieved (88%). This percentage could be obtained because the organic matter present in the sludge was used as carbon source for denitrification. Another cause for this high denitrification percentage could be the incomplete denitrification, producing N_2O instead of N_2. In the rest of the assays, the COD/N ratio was higher than the theoretical value needed for complete denitrification. However, the denitrification process was affected by the presence of formaldehyde. The nitrite accumulation increased with the initial formaldehyde concentration. At the highest formaldehyde concentration (2150 mg l^{-1}), around 100 mg N l^{-1} nitrite accumulated in the medium. Therefore, formaldehyde could be used as carbon source for denitrification but it would be necessary to take into account its concentration since a large amount of nitrite could accumulate.

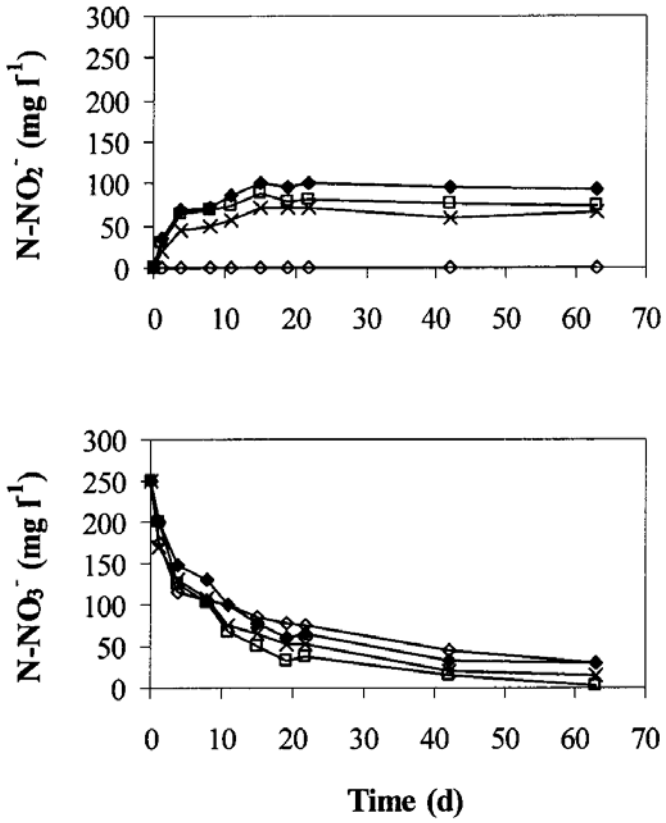

Figure 3. Denitrification process in the presence of different formaldehyde concentrations: (0) 300, (x) 1170, (D) 1550 and (+)2150 mg l^{-1}.

The denitrification process lasted several days, while formaldehyde was completely biodegraded in a few hours. Formaldehyde was transformed into other organic compounds which were then used as carbon sources for denitrification. Products of formaldehyde biodegradation were methanol and formic acid as has been proved in similar assays with formaldehyde and methanol performed in our laboratory [15]. This was also confirmed in the study with the continuous reactor described further in this paper. This is in agreement with results obtained by Kato *et al*. [17] studying aerobic formaldehyde biodegradation by *Pseudomonas putida* F61. They found an enzyme which catalyzed dismutation of formaldehyde to form methanol and formic acid. Adroer *et al*. [18] studied the mechanism of aerobic formaldehyde biodegradation by a strain of *Pseudomonas putida*. Their results indicated that this biodegradation led to the simultaneous appearance of methanol and formic acid. The degradation of methanol and formic acid began after exhaustion of formaldehyde in the medium.

Continuous Reactor

Biodegradation of formaldehyde as the single carbon source

In order to study formaldehyde biodegradation in the continuous reactor and the effect of its concentration on its removal, the amount of formaldehyde in the influent was increased from 625 up to 5000 mg l^{-1}. The evolution of its concentration in the influent and effluent of the reactor is shown in Figure 4.A. The unit remained stable until day 81 of operation, when the formaldehyde and nitrate concentrations in the influent were 4375 mg l^{-1} and 700 mg N l^{-1}, respectively. During this period, high formaldehyde removal efficiencies were reached, with an average concentration in the effluent of 2.2

mg l^{-1}. Removal efficiencies above 98.5% were obtained at all the applied formaldehyde loading rates, between 0.37 and 2.59 kg COD m^{-3} d^{-1}.

Until day 81 of operation, the pH in the effluent was between 7.1 and 8.1, with a mean value of 7.6. Afterwards, a decrease in the pH value was observed down to 4.4, causing the destabilization of the system. The sludge was washed with a buffer solution and formaldehyde and nitrate concentrations in the influent were decreased in order to recover the efficiency of the unit.

After recovering the efficiency, formaldehyde and nitrate concentrations in the influent were again increased up to 5000 mg l^{-1} and 800 mg N l^{-1}, respectively. During this period, the efficiencies were similar to the ones obtained until day 81 of operation. The mean formaldehyde concentration in the effluent was 2.1 mg l^{-1}, reaching removal efficiencies above 99.8% at organic loading rates up to 2.96 kg COD m-3 d-1 (2.78 kg CH_2O m^{-3} d^{-1}).

The formaldehyde loading rates removed in our case (2.78 kg CH_2O m^{-3} d^{-1}) are higher than those reported in the literature. This could have resulted from the long-term (several years) sludge adaptation to formaldehyde in the industrial wastewater treatment plant from which the present inoculum was obtained. Garrido *et al*. [8] studied the treatment of wastewaters from a formaldehyde-urea adhesives factory, using a nitrification-denitrification system in two reactors with suspended biomass. Most of the formaldehyde was used by microorganisms as carbon source for denitrification, obtaining removal rates between 0.2 and 0.6 kg CH_2O m^{-3} d^{-1}. In another study, a multifed upflow filter was used in order to analyze urea hydrolysis, formaldehyde removal and denitrification [9]. The formaldehyde loading rate was progressively increased up to 4.0 kg CH_2O m^{-3} d^{-1}, although the removal rate did not exceed 2.0 kg CH_2O m-3 d-1. Formaldehyde and urea removal was also studied in an anoxic upflow sludge blanket reactor [10]. At the end of the experimental period formaldehyde removal rates of 2.4 kg CH_2O m^{-3} d^{-1} were reached.

Formaldehyde in the influent was easily degraded under anoxic conditions. However, a COD fraction of the influent was present in the effluent of the unit, indicating the possible existence of biodegradation products. As indicated before, formaldehyde removal led to the appearance of methanol and formic acid in the medium (Figure 4.B). Until day 81 of operation, the concentrations of these compounds in the effluent were below 72 mg l^{-1} methanol and 110 mg l^{-1} formic acid. At day 81 of operation, 891mg l^{-1} methanol and 1019 mg l^{-1} formic acid were reached. This high formic acid concentration could be the cause of the pH decrease. Therefore, during the anoxic formaldehyde biodegradation it is necessary to control the operation conditions since a large amount of formic acid could be produced. After recovering the system, the concentrations of both compounds in the effluent decreased; although, the methanol concentration was rather high until the end of the operation. In addition to methanol and formic acid, another organic matter was present in the effluent. The fraction of COD of the influent that was present in the effluent was around 15.4%. This remaining COD in the effluent could be due to extra enzymes or inert bio-products released from the sludge in the reactor caused by turbulent shear stress.

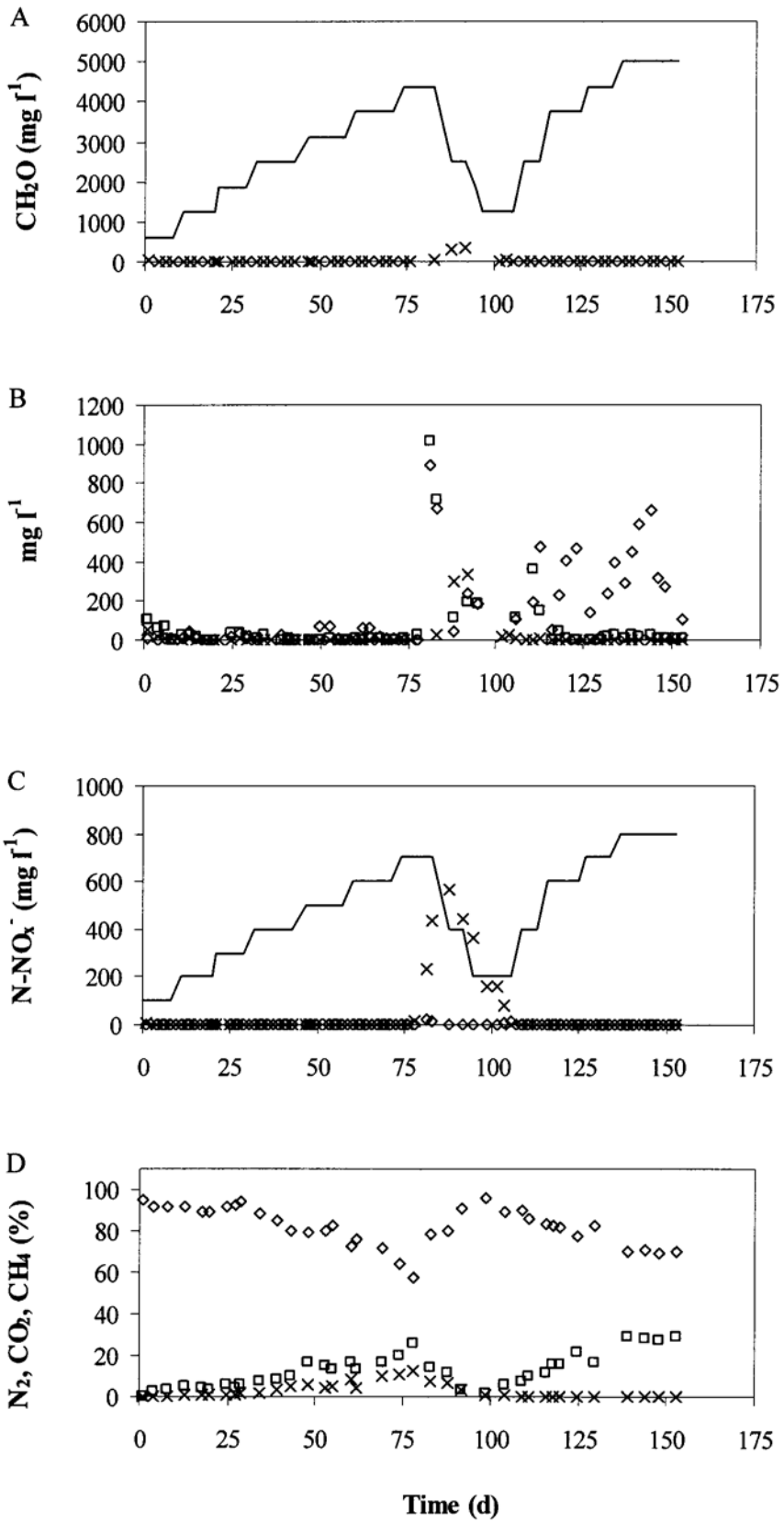

Figure 4. A. Formaldehyde concentration in the influent (—) and effluent (x) of the continuous reactor. B. Concentrations of formaldehyde (x), methanol (◆) and formic acid (□) in the effluent. C. Nitrate in the influent (—) and nitrate (x) and nitrite (◆) in the effluent. D. Evolution of biogas composition: nitrogen (◆), carbon dioxide (□) and methane (x).

Denitrification process in the presence of formaldehyde

Biological denitrification in the presence of formaldehyde was also studied in the continuous reactor. The evolution of nitrate and nitrite concentrations is presented in Figure 4.C. The COD/N ratio was maintained at 6.7 during all the operation period, increasing the nitrate concentration in the influent to between 100 and 800 mg N l^{-1}. Until 81 day of operation, high denitrification percentages were obtained, around 99.7%. But after this day, the decrease in the pH value down to 4.4 caused the destabilization of the system, as has been indicated before. The denitrification percentage decreased to 20.8%, obtaining nitrate concentrations in the effluent of up to 562.8 mg N l^{-1}.

After recovering the efficiency of the unit, the nitrate concentration in the influent was again increased up to 800 mg N l^{-1}. During this period, the denitrification efficiencies were similar to the ones obtained until day 81 of operation (about 99.9%). Therefore, high denitrification percentages were obtained at all the applied nitrogen loading rates, up to 0.44 kg $N\text{-}NO_3^-$ m^{-3} d^{-1}. During all the operation period, nitrite occasionally appeared in the effluent at concentrations always less than 2.9 mg N l^{-1}; except when the pH was low and the nitrite concentration increased to 18.7 mg N l^{-1}.

With the exception of the problem with the pH, no loss of denitrification efficiency was observed when the formaldehyde concentration was increased to a value as high as 5000 mg l^{-1}. Therefore, it can be concluded that formaldehyde was efficiently used as an electron donor for denitrification. This is in agreement with data found in the literature; Garrido *et al*. [8, 9] reached denitrification rates of 1.0 and 0.75 kg $N\text{-}NO_x^-$ m^{-3} d^{-1}, respectively, using formaldehyde as carbon source. Formaldehyde removal was also studied in an anoxic upflow sludge blanket reactor [10]. Formaldehyde concentrations of 250 - 300 mg l^{-1} in that reactor caused a decrease in the denitrification rate. Nevertheless, the denitrification process was totally restored after decreasing the concentration of formaldehyde accumulated. In our study, this inhibition could not be observed since there was no accumulation of formaldehyde in the reactor, except for the pH decrease.

The biogas composition was periodically analyzed as shown in Figure 4.D. Until day 81 of operation, the percentage of nitrogen was decreasing and the percentages of carbon dioxide and methane were increasing. During this period, the percentages of nitrogen, carbon dioxide and methane in the biogas varied between 95.2 - 57.3%, 0.4 - 25.7% and 0 - 12.8%, respectively. From these results it is clear that two processes, denitrification and methanogenesis occurred together in the same unit. Previous studies indicate that if there is enough carbon source in the influent, both processes can occur in the same system [19]. In such case, methanogenesis starts once denitrification has been completed, surplus carbon source is effectively converted to methane and subsequently a low effluent COD concentration is reached [20]. After the pH decrease and when the efficiency of the unit was recovered, the percentages of nitrogen, carbon dioxide and methane in the biogas varied between 89.7 - 69.4%, 7.3 - 29.5% and 0 - 0.2%, respectively. It seems that during the pH decrease, methanogenic bacteria were more affected than denitrifying bacteria.

The biomass concentration in the reactor increased from an initial value of 8 to 11.2 g VSS l^{-1} after five months of operation. No biomass was purged from the reactor throughout the experimental period. The biomass concentration in the effluent increased with the increase in the organic and nitrogen loading rates, ranging between 0.032 and 0.190 g VSS l^{-1} during all the operation time. Sludge with good settling properties and a satisfactory effluent with low concentrations of suspended solids were obtained.

The results obtained in the continuous reactor are in agreement with the results obtained in the batch assays described before. The anoxic biodegradation of high concentrations of formaldehyde and the denitrification using this compound as carbon source are possible. However, unlike batch assays, in the continuous reactor the nitrite accumulation during the denitrification process was very low.

Conclusions

Batch Assays

Formaldehyde was completely degraded at initial concentrations between 300 and 2150 mg l^{-1}. The initial biodegradation rate increased with the formaldehyde concentration, reaching 3.3 g CH2O g VSS^{-1} d^{-1} for the highest concentration.

The denitrification process was affected by the presence of formaldehyde. The nitrite accumulation increased with the initial formaldehyde concentration. At the highest formaldehyde concentration (2150 mg l^{-1}) around 100 mg N l^{-1} nitrite were accumulated.

Continuous Reactor

The formaldehyde concentration in the influent was increased from 625 up to 5000 mg l^{-1}. Removal efficiencies above 98.5% were obtained at all the applied formaldehyde loading rates, between 0.37 and 2.96 kg COD m^{-3} d^{-1}. Formaldehyde removal led to the appearance in the medium of methanol and formic acid.

The nitrate concentration in the influent ranged between 100 and 800 mg N l^{-1}, maintaining the COD/N ratio at 6.7. Denitrification percentages around 99.7% were obtained at all the applied nitrogen loading rates, up to 0.44 kg $N\text{-}NO_3^-$ m^{-3} d^{-1}. Nitrite occasionally appeared in the effluent at concentrations less than 2.9 mg N l^{-1}. The composition of the biogas indicated that denitrification and methanogenesis occurred simultaneously in the same unit.

The results show that the anoxic biodegradation of high concentrations of formaldehyde and the denitrification using this compound as carbon source are possible. However, it is necessary to control the operation conditions in order to avoid the formation of formic acid and nitrite in the effluent.

Acknowledgements

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